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Hyperlipidemia-induced cholesterol crystal production by endothelial cells promotes atherogenesis

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Abstract

© 2017 The Author(s). Endothelial cells (EC) play a key role in atherosclerosis. Although EC are in constant contact with low density lipoproteins (LDL), how EC process LDL and whether this influences atherogenesis, is unclear. Here we show that EC take up and metabolize LDL, and when overburdened with intracellular cholesterol, generate cholesterol crystals (CC). The CC are deposited on the basolateral side, and compromise endothelial function. When hyperlipidemic mice are given a high fat diet, CC appear in aortic sinus within 1 week. Treatment with cAMP-enhancing agents, forskolin/rolipram (F/R), mitigates effects of CC on endothelial function by not only improving barrier function, but also inhibiting CC formation both in vitro and in vivo. A proof of principle study using F/R incorporated into liposomes, designed to target inflamed endothelium, shows reduced atherosclerosis and CC formation in ApoE -/- mice. Our findings highlight an important mechanism by which EC contribute to atherogenesis under hyperlipidemic conditions.

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References

[1] Cahill, P. A. & Redmond, E. M. Vascular endothelium-Gatekeeper of vessel health. Atherosclerosis 248, 97-109 (2016).

[2] Simionescu, M. Implications of early structural-functional changes in the endothelium for vascular disease. Arterioscler. Thromb. Vasc. Biol. 27, 266-274 (2007).

[3] Tabas, I., Williams, K. J. & Boren, J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 116, 1832-1844 (2007).

[4] Gimbrone, M. A. Jr. & Garcia-Cardena, G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ. Res. 118, 620-636 (2016).

[5] Frank, P. G., Pavlides, S. & Lisanti, M. P. Caveolae and transcytosis in endothelial cells: role in atherosclerosis. Cell Tissue Res. 335, 41-47 (2009).

[6] Vasile, E., Simionescu, M. & Simionescu, N. Visualization of the binding, endocytosis, and transcytosis of low-density lipoprotein in the arterial endothelium in situ. J. Cell. Biol. 96, 1677-1689 (1983).

[7] Kraehling, J. R., et al. Genome-wide RNAi screen reveals ALK1 mediates LDL uptake and transcytosis in endothelial cells. Nat. Commun. 7, 13516 (2016).

[8] Armstrong, S. M., et al. A novel assay uncovers an unexpected role for SR-BI in LDL transcytosis. Cardiovasc. Res. 108, 268-277 (2015).

[9] Stewart, G. T. Liquid crystals of lipid in normal and atheromatous tissue. Nature 183, 873-875 (1959).

[10] Hata, Y., Hower, J. & Insull, W. Jr. Cholesteryl ester-rich inclusions from human aortic fatty streak and fibrous plaque lesions of atherosclerosis. I. Crystalline properties, size and internal structure. Am. J. Pathol. 75, 423-456 (1974).

[11] Small, D. M. & Shipley, G. G. Physical-chemical basis of lipid deposition in atherosclerosis. Science 185, 222-229 (1974).

[12] Katz, S. S., Shipley, G. G. & Small, D. M. Physical chemistry of the lipids of human atherosclerotic lesions. Demonstration of a lesion intermediate between fatty streaks and advanced plaques. J. Clin. Invest. 58, 200-211 (1976).

[13] Lim, R. S., et al. Identification of cholesterol crystals in plaques of atherosclerotic mice using hyperspectral CARS imaging. J. Lipid. Res. 52, 2177-2186 (2011).

[14] Janoudi, A., Shamoun, F. E., Kalavakunta, J. K. & Abela, G. S. Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque. Eur. Heart. J. 37, 1959-1967 (2016).

[15] Sheedy, F. J., et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nat. Immunol. 14, 812-820 (2013).

[16] Tangirala, R. K., et al. Formation of cholesterol monohydrate crystals in macrophage-derived foam cells. J. Lipid. Res. 35, 93-104 (1994).

[17] Freigang, S., et al. Nrf2 is essential for cholesterol crystal-induced inflammasome activation and exacerbation of atherosclerosis. Eur. J. Immunol. 41, 2040-2051 (2011).

[18] Rajamaki, K., et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: A novel link between cholesterol metabolism and inflammation. PLoS ONE 5, e11765 (2010).

[19] Duewell, P., et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 464, 1357-1361 (2010).

[20] Vedre, A., et al. Physical factors that trigger cholesterol crystallization leading to plaque rupture. Atherosclerosis 203, 89-96 (2008).

[21] Abela, G. S. Cholesterol crystals piercing the arterial plaque and intima trigger local and systemic inflammation. J. Clin. Lipidol. 4, 156-164 (2010).

[22] Abela, G. S., et al. Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes. Am. J. Cardiol. 103, 959-968 (2009).

[23] Lupu, F., Danaricu, I. & Simionescu, N. Development of intracellular lipid deposits in the lipid-laden cells of atherosclerotic lesions. A cytochemical and ultrastructural study. Atherosclerosis 67, 127-142 (1987).

[24] Spindler, V., Schlegel, N. & Waschke, J. Role of GTPases in control of microvascular permeability. Cardiovasc. Res. 87, 243-253 (2010).

[25] Baumer, Y., Spindler, V., Werthmann, R. C., Bunemann, M. & Waschke, J. Role of Rac 1 and cAMP in endothelial barrier stabilization and thrombin-induced barrier breakdown. J. Cell. Physiol. 220, 716-726 (2009).

[26] Sands, W. A., Woolson, H. D., Milne, G. R., Rutherford, C. & Palmer, T. M. Exchange protein activated by cyclic AMP (Epac)-mediated induction of suppressor of cytokine signaling 3 (SOCS-3) in vascular endothelial cells. Mol. Cell. Biol. 26, 6333-6346 (2006).

[27] Davies, M. J., et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. J. Pathol. 171, 223-229 (1993).

[28] Doseyici, S., Mehmetoglu, I., Toker, A., Yerlikaya, F. H. & Erbay, E. The effects of forskolin and rolipram on cAMP, cGMP and free fatty acid levels in diet induced obesity. Biotechnol. Histochem. 89, 388-392 (2014).

[29] Choi, J. H., et al. Flt3 signaling-dependent dendritic cells protect against atherosclerosis. Immunity 35, 819-831 (2011).

[30] Choi, J. H., et al. Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves. J. Exp. Med. 206, 497-505 (2009).

[31] Jongstra-Bilen, J., et al. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. J. Exp. Med. 203, 2073-2083 (2006).

[32] Paulson, K. E., et al. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. Circ. Res. 106, 383-390 (2010).

[33] Meiler, S., Baumer, Y., Toulmin, E., Seng, K. & Boisvert, W. A. MicroRNA 302a is a novel modulator of cholesterol homeostasis and atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 35, 323-331 (2015).

[34] Singh, R., et al. Autophagy regulates lipid metabolism. Nature 458, 1131-1135 (2009).

[35] Mobius, W., et al. Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway. Traffic 4, 222-231 (2003).

[36] Razi, M. & Futter, C. E. Distinct roles for Tsg101 and Hrs in multivesicular body formation and inward vesiculation. Mol. Biol. Cell 17, 3469-3483 (2006).

[37] Amiya, E., et al. Angiotensin II impairs endothelial nitric-oxide synthase bioavailability under free cholesterol-enriched conditions via intracellular free cholesterol-rich membrane microdomains. J. Biol. Chem. 288, 14497-14509 (2013).

[38] Abela, G. S., et al. Effect of statins on cholesterol crystallization and atherosclerotic plaque stabilization. Am. J. Cardiol. 107, 1710-1717 (2011).

[39] Solomonov, I., Weygand, M. J., Kjaer, K., Rapaport, H. & Leiserowitz, L. Trapping crystal nucleation of cholesterol monohydrate: relevance to pathological crystallization. Biophys. J. 88, 1809-1817 (2005).

[40] Busch, N., Lammert, F., Marschall, H. U. & Matern, S. A new subgroup of lectin-bound biliary proteins binds to cholesterol crystals, modifies crystal morphology, and inhibits cholesterol crystallization. J. Clin. Invest. 96, 3009-3015 (1995).

[41] Kiyotake, R., et al. Human mincle binds to cholesterol crystals and triggers innate immune responses. J. Biol. Chem. 290, 25322-25332 (2015).

[42] Mack, S. G., Cook, D. J., Dhurjati, P. & Butchbach, M. E. Systems biology investigation of cAMP modulation to increase SMN levels for the treatment of spinal muscular atrophy. PLoS ONE 9, e115473 (2014).

[43] Link, A., Selejan, S., Maack, C., Lenz, M. & Bohm, M. Phosphodiesterase 4 inhibition but not beta-adrenergic stimulation suppresses tumor necrosis factor-alpha release in peripheral blood mononuclear cells in septic shock. Crit. Care 12, R159 (2008).

[44] Foey, A. D., et al. Impact of VIP and cAMP on the regulation of TNF-alpha and IL-10 production: implications for rheumatoid arthritis. Arthritis Res. Ther. 5, R317-328 (2003).

[45] Sato, T., et al. Inhibitory effects of group II mGluR-related drugs on memory performance in mice. Physiol. Behav. 80, 747-758 (2004).

[46] Barnard, J. W., et al. Reversal of pulmonary capillary ischemia-reperfusion injury by rolipram, a cAMP phosphodiesterase inhibitor. J. Appl. Physiol. 77, 774-781 (1994).

[47] Hirai, M., et al. Accumulation of liposome with Sialyl Lewis X to inflammation and tumor region: application to in vivo bio-imaging. Biochem. Biophys. Res. Commun. 353, 553-558 (2007).

[48] Hashida, N., et al. High-efficacy site-directed drug delivery system using sialyl-Lewis X conjugated liposome. Exp. Eye. Res. 86, 138-149 (2008).

[49] Tsuruta, W., et al. Application of liposomes incorporating doxorubicin with sialyl Lewis X to prevent stenosis after rat carotid artery injury. Biomaterials 30, 118-125 (2009).

[50] Baumer, Y., et al. CD98 regulates vascular smooth muscle cell proliferation in atherosclerosis. Atherosclerosis 256, 105-114 (2017).

[51] Meiler, S., et al. Selenoprotein K is required for palmitoylation of CD36 in macrophages: implications in foam cell formation and atherogenesis. J. Leukoc. Biol. 93, 771-780 (2013).

[52] Baumer, Y., Drenckhahn, D. & Waschke, J. cAMP induced Rac 1-mediated cytoskeletal reorganization in microvascular endothelium. Histochem. Cell Biol. 129, 765-778 (2008).

[53] Yamazaki, N., Kodama, M. & Gabius, H. J. Neoglycoprotein-liposome and lectin-liposome conjugates as tools for carbohydrate recognition research. Methods Enzymol. 242, 56-65 (1994).